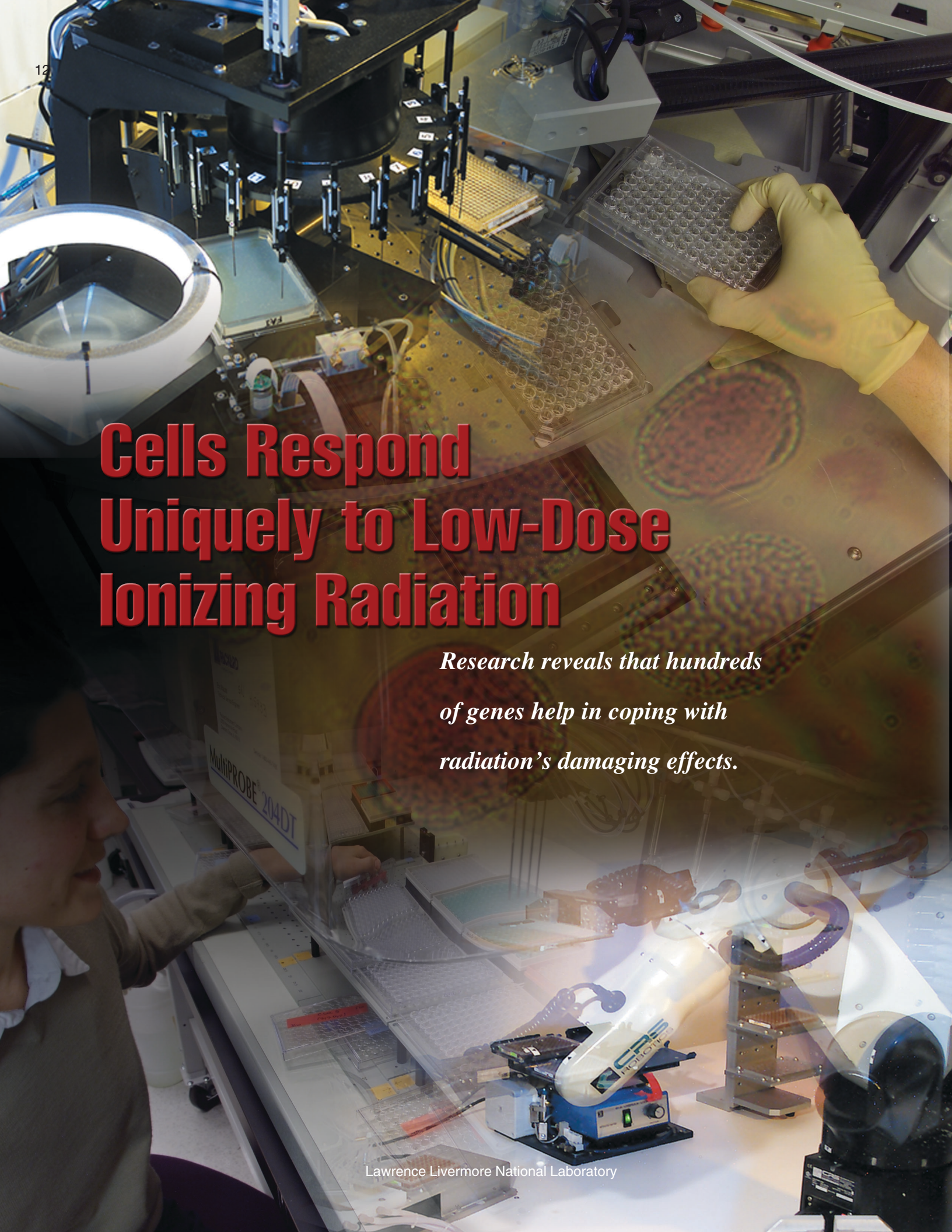


Cells Respond Uniquely to Low-Dose Ionizing Radiation

Research reveals that hundreds of genes help in coping with radiation's damaging effects.

Lawrence Livermore National Laboratory

FOR decades, scientists have studied the cellular and genetic damage that follows exposure to high doses of ionizing radiation such as those resulting from nuclear accidents or cancer radiotherapy. Much less is known about cellular response to low doses of ionizing radiation—about 0.1 gray and below—such as that absorbed by our bodies during medical procedures and normal occupational exposures or while flying in an airplane. (See box on p. 16.)

Research conducted by Lawrence Livermore scientists in the Biology and Biotechnology Research Program (BBRP) Directorate has revealed that cells exposed to low-level ionizing radiation respond in a surprisingly robust manner by turning on or off hundreds of genes, including those specialized in repairing damaged chromosomes, membranes, and proteins and countering cellular stress. These genes involved at low dose are different from the ones responding to high-dose radiation. The discovery that many different genes are called into action only in response to low-dose radiation suggests that a cell's response at low dose involves different functions than those occurring at higher doses.

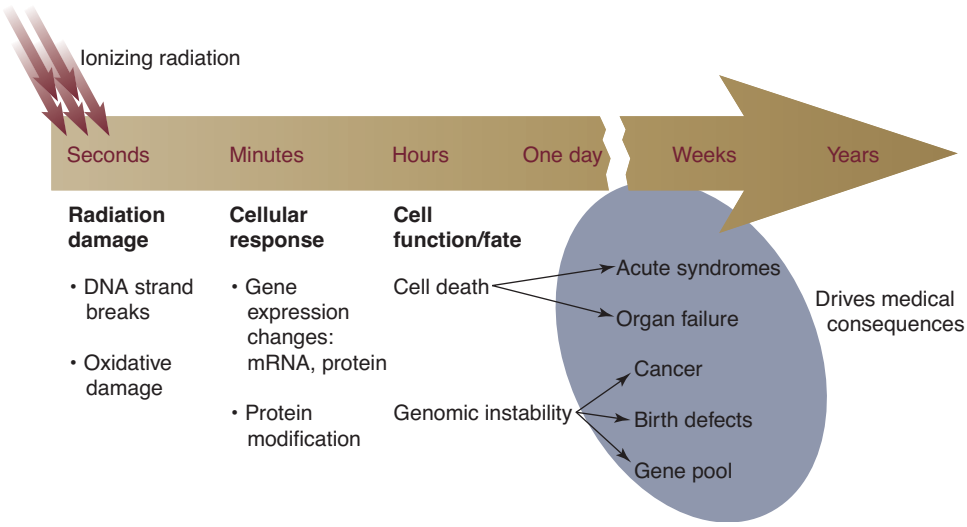
The Livermore research is conducted on tissues of laboratory mice and human cell cultures. The mouse data show different baselines across tissues and specialized responses in irradiated brains. The research in human cells also reveals an intriguing adaptive response, whereby a very small pretreatment dose of ionizing radiation allows the cell to better withstand a later, much higher dose. Similar cellular damage responses may be at work when a cell suffers a low-level insult (injury) from harmful chemicals or is under attack by bacteria or viruses.

The Livermore research team is led by Andy Wyrobek, head of BBRP's Health Effects Genetics Division, and is part of the Department of Energy's Low-Dose Radiation Research Program, which aims to understand the health risks of low-level radiation exposure. This understanding is critical to setting appropriate exposure standards, such as those for people receiving medical tests involving radioisotopes and workers who handle radioactive materials.

Since BBRP's inception in 1963, Livermore researchers have been studying the immediate and long-term health effects of radiation on cells, tissues, and individuals. Livermore-developed techniques, such as chromosome painting and the Glycophorin A and HPRT assays, have been used to monitor genetic damage in Japanese survivors of World War II atomic bomb blasts and in workers cleaning up the Chernobyl nuclear accident. (See *S&TR*, September 1999, pp. 12–15.) Wyrobek says it is well-established that exposure to high doses of ionizing radiation causes physiological, genetic, and chromosomal damage. This damage in turn can cause cell death and increase the risk for later diseases, including cancer and heritable mutations.

However, simply extrapolating from these effects at higher doses to predict changes in cells from low-dose exposure is problematic. Numerous assumptions have traditionally formed the basis for establishing low-level risk, despite the fact that scientists have been unable to directly demonstrate irrefutable health risks from low doses of ionizing radiation.

"We've used high-dose models because, until the past few years, we've been unable to detect cell changes following low doses of radiation," says Wyrobek. Thanks to advances in modern molecular biology and genome instrumentation, much of it developed under the Human Genome Program, this is changing. "We finally have the



An organism's response to ionizing radiation consists of a complex set of physical, chemical, and biological events. Within seconds, radiation produces damage to DNA and oxidizes proteins and DNA, lipids, and other biomolecules. Within minutes, the cell responds by changing the activation of certain genes and modifying some proteins. At high radiation doses, the result may be acute organ failure leading to death or genomic instability that causes cancer and birth defects and affects future generations.

tools to examine the damage response patterns in cells from low doses of ionizing radiation so that we can more scientifically determine health risks from low-dose exposures to ionizing radiation," he says.

Using Mice and Human Cells

To study low-dose cell responses, the researchers are examining the expression profiles of thousands of genes in tissues taken from irradiated adult mice and from irradiated human lymphoblastoid cells (derived from blood-forming cells). The mammalian brain is a relatively radioresistant tissue, while the small intestine and blood-forming tissues are the most sensitive. The team is comparing the findings to control groups of identical cells that received no radiation.

The mouse is an important animal model in radiation biology. Livermore researchers have studied its genome and found surprising similarities to the human genome. (See *S&TR*, May 2001, pp. 14–23.) Mice also provide researchers an opportunity to study many different organs.

The human lymphoblastoid cells were obtained from the National Institutes of Health, which supplies them to researchers nationwide. The cells, originally taken from about 450 adults in the U.S. representing different ethnic backgrounds, are known to be sensitive to ionizing radiation.

Experiments were performed to study the effects of time and dose on gene expression in the mouse brain. A group of mice was irradiated with a 0.1-gray radiation dose from a cesium-137 source, and brain tissue was taken for analysis at 30 minutes and at 4 hours after irradiation. A second mouse group was irradiated with a 2-gray dose (20 times the low-dose radiation and enough to kill some cells), and tissue was sampled 30 minutes and 4 hours later. The same experimental procedure was used for the human lymphoblastoid tissue cells.

The team knew that at higher doses and possibly at low doses of radiation, some genes would respond by modulating their gene expression; that is, they would show either an increase or decrease in messenger RNA (mRNA) or protein levels. (In gene expression, the gene's coded information is converted into mRNA and proteins that are required for cell function and structure.) The researchers examined the populations of mRNA and proteins present in irradiated cells and compared them to mRNA and proteins present in nonirradiated cells as a means to determine whether genes had modulated.

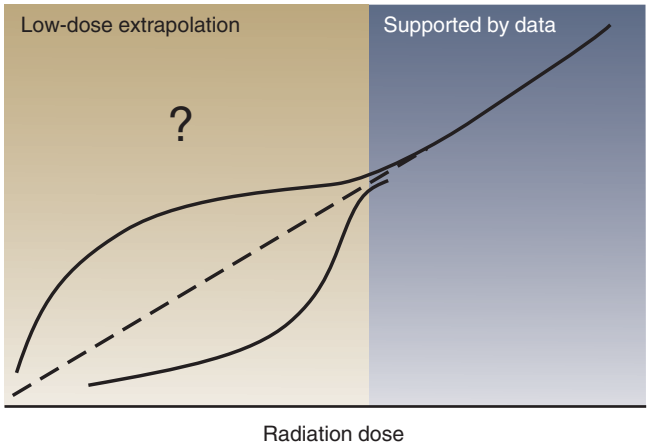
Microarrays Are Key

To simultaneously examine the response of tens of thousands of genes, the team turned to gene-transcript (mRNA) microarray technology, which uses slides or chips containing arrays of up to 20,000 different genes (specific sequences of DNA). The team used both Livermore-manufactured DNA microarrays and commercially available versions.

"Microarray technology allows us to take a nearly global view of what happens to a large number of genes in a cell. It replaces the single-gene approach used in the past," says Wyrobek. He explains that the technology involves labeling pieces of DNA with fluorescent molecules and hybridizing (pairing) them to their complementary DNA target. Much of the fluorescence hybridization technology was pioneered at Livermore and then transferred to private industry.

Following the irradiation step, the team extracted the mRNA from the brain cells, converted it to its complementary DNA (cDNA), labeled that with a fluorescent dye, and applied the fluid mixture to a microarray. The different molecules of cDNA in solution paired with their corresponding genes on the array. The same procedure was done to a control group of cells.

Explains BBRP biomedical scientist Francesco Marchetti, "We can label cDNA from an irradiated cell red and label cDNA from a normal cell green. If we see equal amounts of both red and green for a particular gene, then we know that radiation causes no modulation of that gene. By the same logic, if we see all green, then that particular gene is shut down by radiation. If we see all red, then radiation has switched on that



Scientists have been unable to directly demonstrate irrefutable health risks from low doses of ionizing radiation. As a result, they have made numerous assumptions for establishing the risk.

gene. So the color shifts at each spot on the slide give us information on up to 20,000 genes or more."

Analysis of the microarray generates large volumes of data that require advanced biostatistical and bioinformatics methods. "Fortunately, Livermore is the right place to do these kinds of data-intensive experiments," biomedical scientist Matt Coleman says.

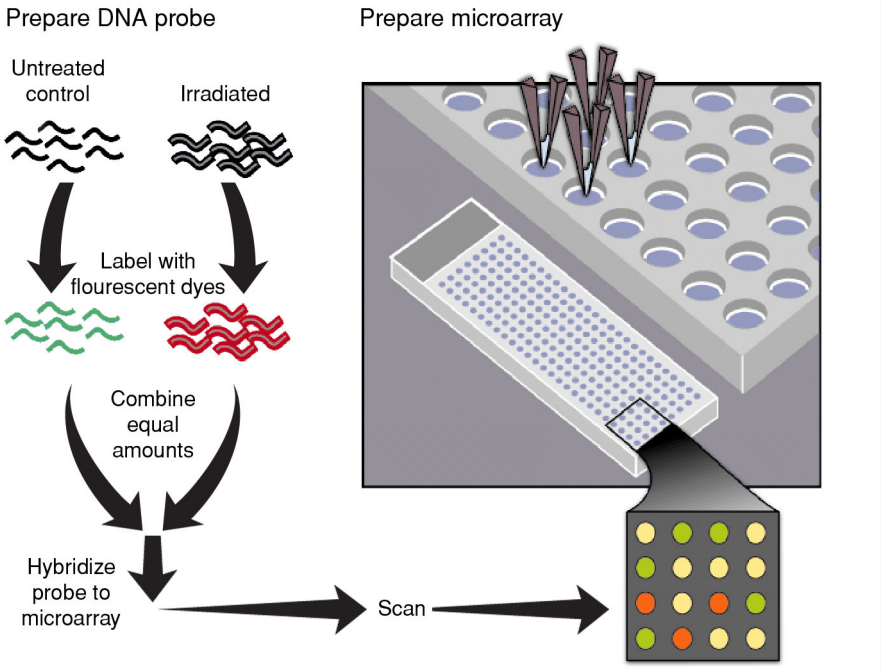
The microarray data are beginning to answer several basic questions the team posed prior to the beginning of the project: Are there genes with differential expression after radiation exposure? Is 0.1 gray enough to elicit gene expression changes in the adult mouse brain? What are the cell functions associated with genes affected by ionizing radiation?

Genes Unique to Low Dose

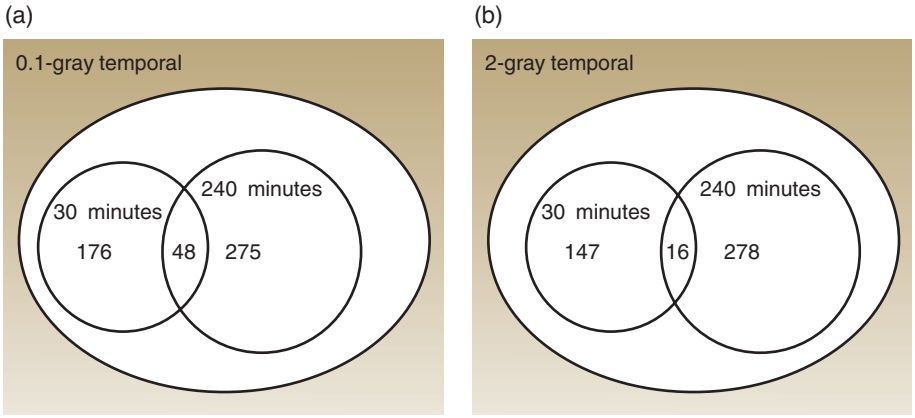
One of the most important findings from the microarray experiments is that cells exposed to a 0.1-gray radiation dose modulate different genes than cells exposed to a 2-gray dose. Likewise, there are also changes over time after exposure; different genes are modulated at 30 minutes and at 4 hours.

The results of one set of experiments involving mice brain cells showed that at a 0.1-gray dose, 176 genes were modulated at 30 minutes and 275 genes were modulated at 4 hours. An overlapping set of 48 genes was time-independent. The genes that are switched on are called REOS genes, or radiation-induced early-onset (within minutes to hours after exposure) and sensitive genes. At a 2-gray dose, 147 genes were modulated at 30 minutes and 278 genes were modulated at 4 hours, with 16 genes being time-independent.

A big surprise was the robust response of cells to ionizing radiation of only 0.1 gray. Says Wyrobek, "When I started this project, I thought we would see very few changes, if any, from such a low dose.



Microarray technology allows the simultaneous examination of tens of thousands of genes through the use of slides or chips. The technology involves extracting all messenger RNA from the irradiated cells, converting it to its complementary DNA, labeling it with a fluorescent dye, and applying it to a microarray. The different molecules of DNA attach to their corresponding genes. The same procedure is done to a control group of cells, but with a different color of fluorescent dye. A laser scans the microarray and analyzes the intensity of the different colors to give information on each gene.



(a) The results of one set of experiments involving mice brain cells showed that at 0.1 gray, 176 genes were modulated (produced more or less messenger RNA) at 30 minutes and 275 genes were modulated at 4 hours. An overlapping set of 48 genes was time-independent. (b) At 2 grays, 147 genes were modulated at 30 minutes, 278 genes were modulated at 4 hours, and 16 genes were time-independent.

"The experiments show that the low-dose response is not simply less than a high-dose response. It's a lot more complicated than that. What is happening here is not linear. For the low-dose extrapolation to be linear, the lower dose would be expected to show less of an effect on expression than the higher dose. But we found many genes where something is uniquely happening in response to low dose—a unique set of genes is getting turned on."

For the mouse brain cells, the genes that modulated exclusively at 0.1-gray appear to be involved in a broad variety of cell functions, including cell-cycle control; DNA, RNA, and protein synthesis and repair; fatty acid metabolism; heat shock; ion regulation; stress response; membrane repair; and myelin (material surrounding nerve fiber) repair. "The list of these pathways suggests that low-dose ionizing radiation may activate protective and repair mechanisms," says biomedical researcher Eric Yin. He notes that low-dose radiation also depresses genes associated with brain signaling activity, probably to divert more resources to repair functions.

The general findings for mouse cells were also seen in the human lymphoblastoid cells, both at the 0.1-gray and 2-gray dose levels. The human tissue cells showed a different set of genes for low-dose response, as might be expected because the cells examined were not brain cells. Wyrobek says it is too early to compare the numbers of genes and the pathways involved between the two kinds of cells because comparable microarrays are not yet commercially available.

Ionizing Radiation: A Short Primer

The broad term radiation includes light and radio waves, but it is often used to mean ionizing radiation. Ionizing radiation has sufficient energy to remove electrons from atoms, thereby creating charged particles (ions or radicals) in materials it strikes. The different kinds of ionizing radiation include neutrons and alpha, beta, gamma, and x radiation. Atoms that emit any of these types of ionizing radiation are radioactive.

The international standard unit of an absorbed dose of ionizing radiation is the gray. One gray is equivalent to the absorption of 1 joule of energy per kilogram of material. It also equals 100 radiation absorbed doses (rads) in the old radiation measuring system. A hundredth of a gray, or one centigray, equals one rad.

Background ionizing radiation levels measure about 0.37 centigray per year, consisting of about 0.3 centigray from natural sources and about 0.07 centigray from sources of human activity. Sources of natural ionizing radiation include radon gas, the human body, rocks and soil, and cosmic rays. Sources of human-caused ionizing radiation include medical procedures, consumer products, and, to a lesser extent, airplane travel, color television, atmospheric fallout from old nuclear tests, and the nuclear power industry.

The occupational exposure limits to ionizing radiation are 5 centigrays per year. Patients undergoing radiation therapy typically receive a daily dose of about 2 grays, with a total dose of about 50 grays or more.

Exposure to large amounts of ionizing radiation can increase the risk of cancer and genetic mutations that can be passed on to future generations. If the dose is large enough, massive cell death can occur as part of acute radiation sickness, which can lead to death. The extent of cell damage depends on the total amount of energy absorbed, the time period and dose rate of exposure, and the particular organs exposed.

Determining exposure limits for workers is an important task for the Department of Energy and other federal agencies. The goal of DOE's Low-Dose Radiation Research Program is to help determine health risks from exposures to low levels of radiation. This information is critical to adequately and appropriately protect people, especially those who are exposed to low levels of ionizing radiation on the job.

Over the next century, experts predict that radiation exposures associated with human activity will be primarily low-dose radiation from medical tests, waste cleanup, terrorism ("dirty" bombs), and environmental isolation of materials associated with nuclear weapons and nuclear power production.

Normal annual exposure from natural radiation (0.3 centigray per year)	
Radon gas	0.20 centigray
Human body	0.40 centigray
Rocks, soil	0.28 centigray
Cosmic rays	0.27 centigray
Normal annual exposure from human-made radiation (0.07 centigray per year)	
Medical procedures	0.0053 centigray
Consumer products	0.0010 centigray
One coast-to-coast airplane flight	0.0002 centigray
Watching color TV	0.0001 centigray
Sleeping with another person	0.0001 centigray
Weapons test fallout	>0.0001 centigray
Nuclear industry	>0.0001 centigray

The chart above shows sources of ionizing radiation from both natural and human sources.

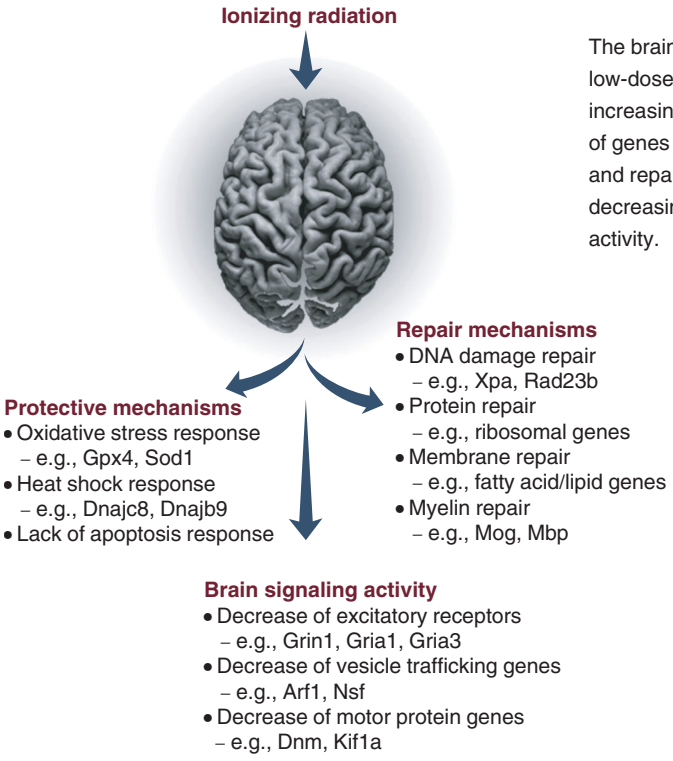
Wyrobek points out that different tissues are expected to respond differently to ionizing radiation. In an experiment of unirradiated tissues, 27 percent of 417 genes represented on a microarray were differentially expressed among five tissues (testis, brain, liver, spleen, and heart). The expression of the DNA repair genes was the least variable among the tissues, while genes responsible for coping with general stress show much greater variability.

Low-Dose Exposure Can Protect

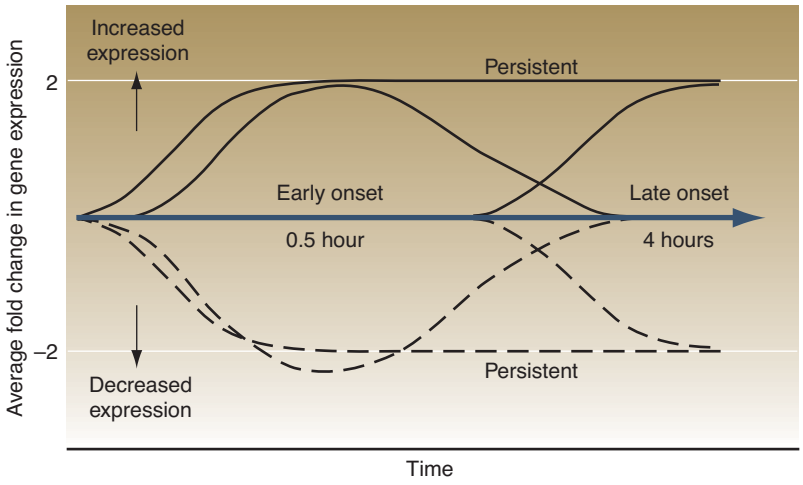
The team also discovered that the human lymphoblastoid cells exhibit what is called an adaptive response to ionizing radiation. An extremely low dose (also called a priming dose) appears to offer protection to the cell from a subsequent high dose (2 grays) of ionizing radiation. The degree of protection was measured by the amount of reduced chromosomal damage. A priming dose of 0.05 gray, administered about 6 hours before the high dose, can reduce chromosomal damage by 20 to 50 percent, compared with damage to cells that were not exposed to the priming dose.

"Pretreatment with a low dose of ionizing radiation sets the cell up to better survive a much higher dose of radiation. A tiny stress apparently helps a cell get ready for a bigger stress," says Coleman. About 200 genes were found to be associated with adaptive response in the human lymphoblastoid cells. Of these, about half were turned on, and half were turned off. "We want to know what genes and pathways are associated with adaptation. Is the adaptive response similar to the low-dose response? We don't yet know."

Coleman says that adaptive responses were first reported in the early 1980s, although many scientists doubted the accuracy of the reports. "Now people are saying this effect happens throughout nature, including in plants. Regulatory agencies are convinced these effects do



The brain seems to respond to low-dose ionizing radiation by increasing expression (activation) of genes involved in protective and repair functions while decreasing brain-signaling activity.



Exposure of the mouse brain to ionizing radiation induces time-dependent changes in gene-transcript (mRNA) expression. Genes associated with specific biological functions show several distinct patterns of radiation response: early-onset and transient, late-onset, and persistent over time. Genes associated with ion regulation and control of gene expression showed early-onset and transient changes. Genes associated with radiation protection (for example, heat shock, oxidative stress) and synaptic signaling showed early onset with both transient and persistent patterns. Genes associated with cellular repair (for example, myelin, protein synthesis) showed late-onset changes in expression.

happen and that they may play a role in human health."

Proteins Provide More Clues

The team is also looking for protein changes in irradiated cells. "Proteins give us a more complete picture of cell response to radiation," says Coleman. However, proteins are more difficult to work with than mRNA because of their instability and many modified forms. "They can go through many reactions that make them active or inactive."

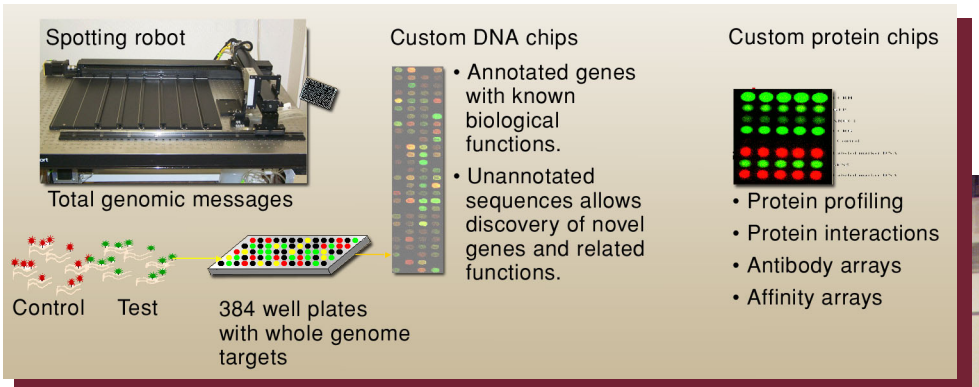
The researchers are using a number of techniques to identify radiation-induced proteins. They are collaborating with colleagues at Livermore and Pacific Northwest Laboratory in using specialized mass spectrometers to gain a better understanding of the proteins. So far, the spectrometers have shown that two proteins, as yet unidentified, seem to be produced in large quantities only in response to high-dose ionizing radiation and are produced in much lower quantities in response to low-dose ionizing radiation.

The team is also using two-dimensional gel electrophoresis, an old and more established technique, to separate and identify proteins. This technology works by separating proteins by their size and electrical charge.

The researchers have also begun using protein microarrays, which work in a similar manner to DNA microarrays. The value of this technique is limited, however, because users must know in advance what proteins they are trying to find.

More Work Ahead

Much work lies ahead. "We still have to show exactly what cell mechanisms and pathways come into play. We need to identify the genes that are expressed in association with a low-dose ionizing radiation exposure and those that are expressed for adaptive response," says Wyrobek. The team also needs to better



Researcher Chitra Manohar shows colleague Hitesh Kapur the analytical results of changes in gene expression of tens of thousands of genes examined by microarray technology. The microarrays are on the spotting robot to the right of the computer screen. Livermore has developed customized microarrays for analyzing specific sequences of DNA and proteins (see inset).

understand the differences among tissues and how these relate to the risk of acute radiation sickness and long-term health effects.

The DOE low-dose program is also preparing to study low-dose response from other chemical and microbial toxins. "These radiation effects studies are setting the stage for modern molecular toxicology of cells and tissues," says Wyrobek.

"Radiation is just one kind of toxic material that damages chromosomes and kills cells. Does a cell respond in like manner to toxic chemicals, bacterial toxins, or even to cell toxicity caused by bacteria or viral infections? We don't know. We do know that some of the genes involved in cell response to low-dose ionizing radiation are the same ones that respond to chemical stress and to viral and bacterial infection." The answers to all the radiation-response questions may have a huge effect on understanding whether high doses of a suspected toxic chemical on laboratory animals are relevant to humans ingesting the same material but in much smaller doses.

Another worthwhile avenue of research is determining if individual genetic differences exist that render some people more or less sensitive to ionizing radiation. Wyrobek notes also that the Livermore experimental findings are based on the aggregates of millions of cells. "It is possible, for example, that just one or only a few types of cells within a tissue can respond differently to ionizing radiation. We already know that cells in tissues differ dramatically in their sensitivity to cell killing, but we know little about the underlying molecular mechanisms. Determining the differential response of cells in tissues to insult is an important next step of research."

An important new research tool available to Livermore researchers is a nanoscale dynamic secondary-ion mass spectrometer (NanoSIMS). This instrument is only the second such machine in the nation dedicated to biological research. It can scan a tissue and identify the regions where a selected gene is expressed. (See p. 18 of the previous article for a discussion of NanoSIMS used for quantitative imaging of biological materials.)

Wyrobek says the experimental findings are relevant to homeland security and for assessing biological dose after incidents of chemical and biological warfare and so-called dirty radiological bombs. Whenever there is a suspected exposure incident, investigators will always have to try to determine exposure dose and assess health effects.

He notes that it is too early to tell if exposure standards will be changed as a result of the work funded by the DOE low-dose program. "We know a lot of things are going on at the low-dose level. What they all mean in terms of health is uncertain," says Wyrobek. But the new knowledge will certainly help ensure that the existing standards are appropriate. At the very least, he says, "We should no longer assume that cells respond in a linear fashion to exposure to ionizing radiation."

—Arnie Heller

Key Words: DNA, human lymphoblastoid cells, ionizing radiation, Low-Dose Radiation Research Program, messenger RNA (mRNA), nanoscale dynamic secondary ion mass spectrometer (NanoSIMS), radiation-induced early-onset and sensitive genes (REOS).

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